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Responses of the Murine Myeloid Colony-Forming Cell to Ansamycin Antibiotics

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The in vitro susceptibility of murine myeloid colony-forming cells to the antiproliferative activities of three ansamycin antibiotics was determined. These cells were found to be 10- to 40-fold more susceptible than the corresponding human ones.

Streptovaricins and rifamycin SV (RiSV) derivatives are members of the ansamycin family which display antimicrobial activity against many gram-positive and some gram-negative bacteria (8). Additionally, antiviral effects against bacteriophages (5), poxviruses (7), and murine oncornaviruses (4) have recently been demonstrated. With ribonucleic acid (RNA) tumor viruses, the mechanism of ansamycin-induced inhibition may be related to the blockage of the virion-associated RNA-dependent deoxyribonucleic acid (DNA) polymerase (3, 11). Ansamycins block RNA tumor virus function at several levels of organization, including tissue culture systems (4) as well as the whole animal (2). For example, we have reported (2) a 50% inhibition of Rauscher leukemia virus-induced splenomegaly in mice fed sufficient streptovaricin complex (SvCx) to result in a relatively steady serum concentration of 1 to 3 μg/ml. At these drug concentrations, no effects on the cloning of normal syngeneic cells (primarily *erythropoietic* in nature) in the spleens of mice can be seen (2). Also, induction of murine splenomegaly by multiplication of injected L1210 lymphoma cells proceeds unchanged; primary and secondary antibody responses can develop normally in animals receiving 100 mg of SvCx per kg of body weight daily; finally, interferon induction (by $rI_n \cdot rC_n$ and Newcastle disease virus)—as well as interferon action—proceeds normally (our unpublished data). In addition, those ansamycins capable of inhibiting the RNA-dependent DNA polymerase of oncornaviruses show selective toxicity for the human leukemic "blast" cell when it is maintained in short-term culture (9).

Taken collectively, these observations suggest pronounced specificity in the action of the ansamycin molecule, at least at certain drug concentrations and under certain experimental conditions.

We have determined the effect of these new antitumor compounds on additional hematopoietic elements of mice, and now report in vitro studies on the antiproliferative activities of SvCx, streptovaricin C (SvC), and RiSV on the murine *myeloid* colony-forming cell (CFC). Murine CFCs can give rise to colonies, or clusters of cells, in vitro when a soft agar cell culture system is employed (6).

Cell suspensions prepared in collecting fluid (CF) (6) were derived from the femoral bone marrow of six 9-week-old DBA/2J and Swiss mice. The bone marrow cells were then added to an agar medium described by Metcalf (6) to yield a final cell concentration of 3.3×10^4 cells/ml. As a source of colony-stimulating "factors," "conditioned" medium derived from L929 cells was used (10% vol/vol) (1). Portions (1.5 ml) of the cell suspension were dispensed (in sextuplicate) in FB-6 tissue culture plates (Linbro) and solidified at room temperature. SvC (lot no. 7223WMH60-1) and SvCx (lot no. 11560-5) were generously supplied by the Cancer Chemotherapy Branch of the Upjohn Company (Kalamazoo, Mich.), RiSV (sodium salt) was obtained from Schwarz-Mann, (Rockville, Md.). These drugs were dissolved, immediately before use, in CF and appropriately diluted before addition (0.5-ml portions) to the CFC cultures. After 6 days of incubation at 37 C (humidified 7.5% CO_2 atmosphere), colonies were counted after being stained for 60 min with methylene blue in phosphate-buffered saline (0.5 ml of a 1:3000 (wt/vol) solution). A dissecting microscope (100 × magnification) was used, and any focus containing over 40 cells was scored as a "colony."

We first conducted experiments to detect any association between antibiotic and the solid agar support phase which might change the concentration of drug available for interaction with CFC. SvC (10 or 40 μg/ml) was mixed with 0.6% agar (in phosphate-buffered saline, pH 7.2, 40 C); the mixture was solidified and held at 37 C for 60 min. It was then homogenized and centrifuged, and the concentration of SvC present in the supernatant fraction was determined spectrophotometrically. No significant formation of streptovaricin-agar complexes could be detected.

Murine CFC cultures were then studied with various doses of SvCx, SvC, and RiSV (Table 1). In the control plates (without drugs) the mean number of colonies (per 5×10^4 cells seeded) were: 48 for DBA/2J and 44 for Swiss mice. For both strains of mice, all three ansamycins displayed very strong anti-CFC activity at concentrations of less than 10 μg/ml. The order of potency was RiSV > SvCx > SvC. Mean 50% inhibitory concentrations were 0.25, 0.8, and 4.5 μg/ml, respectively, for DBA/2J mice, and 0.24, 0.6, and 4.8 μg/ml, respectively, for Swiss mice; slopes of the dose-response curves were similar. We note that the structure-activity relationship for antiproliferative activity is very dissimilar to the structure-activity relationship for antitranscriptase activity (3). For example, SvCx, a more potent inhibitor of the viral enzyme than RiSV, is about threefold less active in blocking CFC proliferation.

Importantly, our current results delineate the unusual sensitivity of the murine *myeloid* CFC to this class of antiviral compounds; we have previously shown that the in vivo cloning efficiency of the murine *erythroid* CFC is uninfluenced by two- to five-fold greater serum antibiotic concentrations (2). This apparent enhancement in drug susceptibility of one cell species within the hematopoietic compartment, at least when studied in vitro, suggests that similar effects might be incurred in vivo. Such effects could partially undermine the apparent specificity of action for viral-induced function(s).

Recently we have determined (J. S. Horoszewicz et al., J. Nat. Cancer Inst., in press) the in vitro effects of SvCx, SvC, and RiSV on human CFCs in a similar soft-agar culture system. We studied the peripheral blood of 19 normal individuals and two patients with chronic myelogenous leukemia. All three ansamycins, each of which inhibits the RNA-dependent DNA polymerase of RNA tumor viruses, displayed strong anti-CFC activity at concentrations less than 50 μg/ml. The order of potency was RisV > SvCx > SvC, and the mean 50% inhibitory concentrations were 2.5, 25, and 42 μg/ml, respectively. We observed similar antiproliferative activities of these ansamycins on the CFCs from both chronic myelogenous leukemia patients and normal subjects.

TABLE 1. *Effects of rifamycin SV, streptovaricin complex, and streptovaricin C on colony formation by normal murine colony-forming cells*

Ansamycin	Concn (μg/ml)	% of Control values	
		DBA/2 mice	Swiss mice
Rifamycin SV	0.5	4	5
	0.4	7	5
	0.3	37	27
	0.2	70	67
Streptovaricin complex	1.5	2	7
	1.0	43	18
	0.5	70	58
	0.25	77	68
Streptovaricin C	10.0	0	0
	7.5	9	11
	5.0	46	47

In this report, we have noted that a *comparison* of myeloid CFCs of murine and human origin reveals an apparent greatly increased susceptibility (10- to 40-fold) of the murine cell to the antiproliferative activity of the ansamycin molecule. In addition, the murine myeloid CFC is apparently a more susceptible cell species than the murine erythroid CFC. The enhanced susceptibility might be related to the widely recognized ubiquity of RNA tumor viruses in the mouse (10). Studies are now underway to determine if the order of CFC reactivity to this family of compounds is indeed related in some fashion to the number of RNA tumor virus transcripts.

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